Diversity and the Composition of Fatty Acids of Lipolytic Bacteria Isolated from Soil and Aquatic Sediment in a Forest and on an Oil Palm Plantation

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Diversity and the Composition of Fatty Acids of Lipolytic Bacteria Isolated from Soil and Aquatic Sediment in a Forest and on an Oil Palm Plantation

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Abstract

The specific bacteria in forests and on oil palm plantations are lipolytic bacteria. Their enzymes have been applied in the agro-aquaculture, food, detergent, pharmaceutical, dairy, and biodiesel-biokerosene industries. This study describes the diversity of cultivable lipolytic bacteria from soil and aquatic sediment in a forest and on an oil palm plantation and their fatty acid products. Soil samples used in this research were obtained from topsoil in a lowland forest and on an oil palm plantation and from sediments in fresh water near these sites. The forest is located in Bukit Duabelas National Park, and the oil palm plantation is near the forest in Sarolangun District, Jambi Province, Indonesia. Twenty-two isolates of lypolitic bacteria were selected from 32 isolates grown in lipolytic selective medium. The 22 consisted of 11 isolates from topsoil and 11 from aquatic sediment from the forest and plantation area. These isolates were identified by 16S rRNA-sequence data analysis. Taxonomically, they belonged to five genera: *Burkholderia, Cupriavidus, Serratia, Acinetobacter,* and *Kurthia*. The maximum likelihood tree showed that they are phylogenetically distributed in three clusters. They were clustered into three groups: the *Burkholderia-Cupriavidus* group, the *Serratia-Acinetobacter* group, and the *Kurthia* group. Their lipolytic enzymes formed various fatty acids after analysis by gas chromatography-flame ionization detector (GC-FID). Some isolates formed essential fatty acids, such as linoleic, linolenic, arachidonic, eicosapentanoic acid (EPA), and docosahexanoic acid (DHA).

Abstrak

Keragaman dan Komposisi Asam Lemak Bakteri Lipolitik Isolat Asal Tanah dan Sedimen Perairan Wilayah Hutan dan Perkebunan Sawit. Bakteri spesifik dalam hutan dan perkebunan kelapa sawit adalah bakteri lipolitik. Enzim-enzim dari bakteri lipolitik telah diaplikasikan di agro-akuakultur, makanan, deterjen, farmasi, industri susu, dan biodiesel-biokerosin. Penelitian bertujuan memberikan informasi keragaman bakteri lipolitik asal tanah dan sedimen perairan di areal hutan dan perkebunan kelapa sawit, dan komposisi asam lemaknya. Sampel tanah berasal dari tanah lapisan atas hutan dataran rendah dan perkebunan kelapa sawit dan sedimen air tawar di dekat situs tersebut. Hutan tersebut terletak di Taman Nasional Bukit Duabelas dan perkebunan kelapa sawit sekitarnya di Kabupaten Sarolangun, Jambi, Indonesia. Sebanyak 22 isolat terpilih dari tiga puluh dua isolat bakteri lipolitik yang tumbuh pada media selektif lipolitik, terdiri dari 11 isolat dari tanah lapisan atas dan 11 isolat dari sedimen air di hutan dan daerah perkebunan. Isolat-isolat bakteri diidentifikasi berdasarkan analisis gen 16S rRNA. Hasil identifikasi menunjukkan isolat-isolat tersebut terdiri atas lima genera yaitu *Burkholderia, Cupriavidus, Serratia, Acinetobacter,* dan *Kurthia*. Pada pohon filogenetik yang dibangun menggunakan metode maximum likelihood isolat-isolat tersebut terdistribusi ke dalam tiga grup, yaitu grup *Burkholderia-Cupriavidus*, grup *Serratia-Acinetobacter*, dan grup *Kurthia*. Hasil analisis kromatografi gas (GC-FID) menunjukkan bahwa enzim lipolitik yang dihasilkan bakteri-bakteri tersebut terdiri atas berbagai asam lemak. Beberapa isolat bakteri menghasilkan asam lemak esensial, seperti asam lemak: linoleat, linolenat, arakidonat, eikosapentanoat (EPA), dan dokosaheksanoat (DHA).

Keywords: aquatic sediment, forest, lipolytic bacteria, oil palm plantation, soil

1. Introduction

Between 1999–2006, 3.5 million hectares of Indonesian forest were damaged per year. Between 2006–2010, 2 million hectares per year were damaged, and between 2010–2012, 300,000 hectares per year. In Jambi Province, Sumatra, Indonesia, land has been transformed from forest to temporary cropland and agro-forest [1]. Loss of

environmental services provided by forests is a non-linear process in this province. A gradual simplification of complex agro-ecosystems to agro-forests with increased profitability might be threatened by the oil palm plantation industry [2].

Tropical, lowland forests are ecosystems rich in endemic species of flora, fauna, and microbes like prokaryotes, a species of bacteria. Specific prokaryotes are lipolytic bacteria, which are important for most nutrient transformations in soil and are major drivers of bio-geochemical cycles in oil palms. Lipolytic bacteria have been found in forest grass and soil [3], marine sediment [4, 5], river surfaces [6], hot spring water [7], agricultural industry waste [8], soil contaminated by wastewater treatment [9], and endophytic lipolytic bacteria of palm oil fruit [10]. Fallen palm oil fruit and debris from the cell membranes of dead animals have provided lipolytic bacteria substrates for degrading and synthesizing many kinds of fatty acids in the soil of oil palm plantations and forests. Lipolytic bacteria secrete lipase, which is used by many industries. The majority of lipase currently used industrially has been isolated from cultivated microbes.

Bacteria produce and secrete lipases, which can catalyze both the hydrolysis and the synthesis of long-chain acylglycerols. Lipolytic bacteria produce extracellular lipases in their environments. These enzymes are capable of hydrolyzing and synthesizing carboxyl esters of long-chain acylglycerol (≥ 10 carbon atoms). They have applications in various industries, such as food, detergent, pharmaceutical, dairy, and biodiesel-bio-kerosene [11, 12]. They can be developed in sustainable agro-aquaculture if the applications are cheaper than those used in industry. Lipases are produced by various organisms, including animals, plants, fungi, and bacteria. Bacterial enzymes are easier to manipulate in culture. Commercially, lipases from bacteria have been produced by *Burkholderia cepacia, Pseudomonas mendocina, Pseudomonas alcaligenes,* and *Chromobacterium viscosum* [11]. This study describes the diversity of cultivable lipolytic bacteria from soil and aquatic sediment in a forest and in the area of an oil palm plantation, their capability for producing lipases, and their fatty acid composition.

2. Methods

Soil and sediment sampling. Soil samples used for this research were derived from the topsoil of a lowland forest and an oil palm plantation and from sediment in fresh water near these sites. The forest is located in Bukit Duabelas National Park, and the oil palm plantation is near the forest in Sarolangun District, Jambi Province, Indonesia.

Notations used on the samples were BF1, BF3, and BF4 for the lowland forest area, and BO2, BO3, and BO4 for

Figure 1. Map of Sampling Location from Lowland Forest (Bukit Duabelas National Park) and Oil Palm Plantation at Sarolangun, Jambi Province, Sumatra, Indonesia (between 102°32'00''–102°45'00'' East and 1°550'–2°50' South) [39]

the oil palm plantation area. Samples were collected in December 2012. In each of these six subareas, small cores were collected in five replicates and triplicates of each core (approximately 10 grams) from topsoil and surface sediment (approximately 5 cm from the top), using a small shovel and tube (approximately 2 inches in diameter) (Figure1).

Acidity of soil and sediment was measured with a pH meter for soil in situ. All samples were maintained on ice until arrival at the laboratory and then were stored at 4°C. Samples from the same area were combined and mixed completely to produce one homogeneous sample. These samples are BF (soil from block of lowland forest), BO (soil from block of oil palm plantation), SBF (aquatic sediment from block of lowland forest), and SBO (aquatic sediment from block of oil palm plantation).

Isolation of cultivable lipase-producing bacteria. Soil and sediment were serially diluted with NaCl 0.85% solution to 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6}. Bacteria in this solution were spread on a selective medium with olive oil. The medium used for isolation and screening contains 2.5% olive oil (w/v); 0.2% glycerol (w/v); 0.002% rhodamine B, Merck (w/v); 1.5% peptone (w/v); 0.2% KH_2PO_4 (w/v); 0.01% $MgSO_4.7H_2O$ (w/v); 0.5% NaCl (w/v); 0.5% yeast extract (w/v); 3.0% agar (w/v); and 0.1% cycloheximide (w/v) [10]. The medium was adjusted to pH 5.5 with HCl 0.2 M. Plates are incubated at 28 °C for 3 d. Colonies on the plates were coded as BF, BO, SBF, and SBO. Colonies with orange fluorescent halos around them were purified by repeated streaking on the selective medium agar plates, and the pure culture of the single colony was picked out for lipase production in liquid medium, without agar and rhodamine B.

Lipase product assay using GC-FID. Cell culture samples (5 mL) were centrifuged at 4 °C and 12,000 x g for 30 min, and the supernatants were assayed for lipase activity after NaCl saturation of each supernatant of solution. Two milligrams of recovered lipids are reconstituted in 1 mL of 0.5 M methanolic KOH and hydrolyzed at 80 °C for 1 h. Then, 1 mL of fresh 10% BF3 in methanol was added. Trans-esterification was performed at 100 °C for 20 min. After trans-esterification, 2 mL DI H_2O and 1 mL hexane were added to the sample to quench the reaction. The recovered organic phase was pooled and spiked with methyl ester of 21:0 to a final concentration of 25.0 µg/mL. Products of reactions were determined using a standard fatty-acid methyl-ester mixture (FAME Mix) (10 mg FAME Mix per dichloromethane) by GC (Gas Chromatography) methods. An Agilent Technologies 6890 Gas Chromatograph with Auto Sampler, a 6890 Flame Ion Detector, and a Chemstation data system were used. The column was an HP Ultra 1 capillary column with length (m) 50 X 0.200 (mm) I.D. X 0.11 (µm) film thickness. Ovens used an initial temperature of 160 °C for 2 min, rose at 2 °C/min to 240 °C, rose at 10 °C/min to 290 °C and held for 10 min. Injection port, detector, and inlet temperatures were 250 °C, 250 °C, and 260 °C, respectively. Carrier gas was Helium. The column mode was constant flow 1.5 µL/min., and the injection volume was 5 µL.

DNA sequencing of 16S rRNA gene. Cells grown on broth selective medium (pH 6–7) for 24 h were harvested by centrifugation (10,000 x g, 3–5 min). The supernatant was discarded, leaving approximately 25 µL of liquid. The pellets were resuspended in mixer for 10 sec. Solution DNA was purified with Master Pure™ DNA Purification Kit (EPICENTRE, Madison, WI) according to the manufacturer's protocol. The DNA was resuspended in 35 mL of TE buffer. A PCR amplifying the nucleotide of the *Escherichia coli* 16S rRNA gene was carried out with the forward primer, 341F, and the reverse primer, 1114 R. In a total volume of 50 µL, 30 µL DEPC-treated water and 2.5 µL DNA template were added [13]. PCR products were purified with a QIA quick spin kit (QIAGEN, Hilden, Germany) and were sequenced directly with a SequiTerm Excel II LC DNA sequencing kit (Epicentre Technologies, Madison, WI). An automated, infrared laser fluorescence sequencer (Model 4000 DNA Sequencer; Li Cor, Lincoln, NE) was used for sequencing. Sequences of each sample were analyzed by a BLAST homology search tool [13].

Phylogenetic analysis. The Ribosomal Database Project and the BLASTN 2.0.6 algorithm of GenBank were employed to search for close evolutionary relatives of the 16S rRNA gene sequences of bacterial isolates. Alignments were generated with ClustalW. The partial 16S rRNA genes were sequenced at two different sites, forward and reverse, with an overlap between the

sequences. Therefore, it was possible to align both sequences from the same isolate to get a longer fragment of the 16S rRNA region. Aligning the two sequences led to a consensus sequence. Furthermore, false base pairs could be edited and exchanged, or added, if there were missing base pairs. The consensus sequence was copied and compared with an NCBI database sequences from isolates and reference sequences from known strains, and sequences from the results of the NCBI search were aligned with the Aligner program from http://www.arb-silva.de/ (http://www.arb-silva.de/aligner/). The phylogenetic tree was constructed with the MEGA5 program by using Maximum Likelihood methods. Bootstrapping [14] was used to estimate the reliability of phylogenetic reconstructions with 1,000 replicates.

3. Results and Discussion

Lipolytic bacterial isolates obtained from forest and oil palm plantation. There were 32 isolates of soil and aquatic sediment from the forest and the oil palm plantation, grown on lipolytic bacteria selective medium. Preliminary data showed that 22 isolates were growing on purely olive-oil lipolytic bacteria medium. Rhodamine B medium caused formation of a lytic zone around the colony of lipolytic bacteria in one qualitative assay. Isolates of lipolytic bacteria were separated according to the source of the sample, topsoil from the forest and the oil palm plantation accounted for 6 and 5 isolates, respectively, and aquatic sediment from the forest and the oil palm plantation accounted for 4 and 7 isolates, respectively. The lipolytic bacteria from sediment in water in the forest and at the oil palm plantation showed more genera compared that from top soil (Table 1).

Three genera of bacteria, *Burkholderia*, *Serratia*, and *Kurthia*, were found in forest samples. Three genera of bacteria, *Burkholderia*, *Cupriavidus*, and *Acinetobacter*, were found in oil palm plantation samples. *Burkholderia* was found in both forest and plantation. This genera also dominated in both topsoil and aquatic sediment habitats. Isolates of cultivable lipolytic bacteria from topsoil in the forest area (BF) and in the oil palm plantation area (BO) were dominated by *Burkholderia* (Table 1). Members of this genus are also found in a variety of ecological niches. They inhabit humid areas, industrial zones, and the rhizosphere, existing in symbiosis with various plants and mushrooms [16]. The gram-negative, rod-shaped bacteria were found in acidic forest soil [17] and burned forest soil [18]. The soil in the forest is acidic soil, with a pH value of 3.1–5.0. Oil palm plantation soil also was acidic, with an average pH value of 5.0. The plantation area is suspected to be a burned forest area.

Initial purification of bacterial lipolytic isolates showed that lytic zones indicate the diversity of bacterial lipolytic

Table1. Isolates of Lipolytic Bacteria from Forest and Oil Palm Plantation Soil and Aquatic Sediment and Their Lytic Zones

Code of isolates: Taxon assignment	Identity (%)	Diameter of lytic zone (mm)
BF01: *Burkholderia* sp.	99	7.75 ± 0.35
BF02: *Kurthia* sp.	100	nd
BF03: *Burkholderia cepacia*	99	6.25 ± 0.35
BF04: *Burkholderia* sp.	99	7.50 ± 0.71
BF06: *Burkholderia oxyphila*	99	nd
BF11: *Burkholderia* sp.	99	7.25 ± 0.35
BO01: *Burkholderia cepacia*	100	6.00 + 1.41
BO03: *Burkholderia* sp.	100	7.50 + 0.00
BO04: *Burkholderia* sp.	99	7.25 + 0.35
BO11: *Burkholderia vietnamiensis*	99	7.50 + 1.41
BO12: *Burkholderia pyrocinia*	100	6.00 ± 0.00
SBF02: *Burkholderia* sp.	99	3.75 ± 0.35
SBF11: *Serratia* sp.	99	17.00 ± 1.41
SBF12: *Serratia* sp.	99	15.75 ± 0.35
SBF15: *Burkholderia* sp.	99	nd
SBO01: *Burkholderia* sp.	99	14.00 ± 0.71
SBO02: *Burkholderia* sp.	99	7.50 ± 0.71
SBO03: *Acinetobacter* sp.	98	nd
SBO04: *Acinetobacter* sp.	99	10.25 ± 2.47
SBO06: *Cupriavidus* sp.	99	5.25 ± 1.06
SBO11: *Acinetobacter* sp.	98	7.50 ± 0.71
SBO12: *Acinetobacter* sp.	99	nd

Note: nd = none determined (isolates could grow on lipolytic medium, but their lytic zone was < 0.5 mm)

capabilities. It could not be detected that some isolates even had lytic zones, as was the case with isolates BF02, BF06, SBF15, SBO03, and SBO12. Isolates SBF11, SBF12, SBO01, and SBO04 had formed lytic zones of more than 10 mm. These isolates came from aquatic sediment. The diameters of lytic zones in isolates from the forest were greater than those from the oil palm plantation.

Phylogenetic analysis of 22 identified isolates of lipolytic bacteria resulted in three clusters (Figure 2). The first cluster is dominated by all bacterial isolates closely related to *Burkholderiales* in the forest and oil palm plantation area, including in the topsoil and aquatic sediment. This cluster consisted of the genera *Burkholderia* and *Cupriavidus*. The second cluster consisted of the genera *Serratia* and *Acinetobacter*. Those isolates closely related to the genus *Serratia* were acquired from forest aquatic sediment (Table 1), whereas those closely related to *Acinetobacter* were acquired from oil palm plantation aquatic sediment. The third cluster consisted of only one Bacillales, the genus *Kurthia*. The isolate *Kurthia* BF02 was obtained from forest soil.

The first cluster of phylogenetic tree consisted of isolates closely related to the genus *Burkholderia,* and only isolate SBO06 was closely related to the genus *Cupriavidus* (Figure 2). *Burkholderia* is a phylogenetically coherent genus within a beta-proteobacterial subphylum first described by Yabuchi (1992) [19] by transferring seven species from the genus *Pseudomonas*. If they were isolated from their environment, they may have formed non-pathogenic associations with plants, such as *Burkholderia cepacia*. In recent years, new *Burkholderia* species have been reported as nitrogen fixing, non-pathogenic, plant growth promoting bacteria, and have been proposed as candidates for biotechnological applications [19]. *Burkholderia* species are producers of lipolytic enzymes. Lipolytic enzymes, which have been produced by *Burkholderia cepacia* and *Burkholderia glumae*, could be classified in the lipolytic family 1.2 (family 1 and subfamily 2) as true lipases [23].

In the first cluster, the isolate SBO06 is closely related to the genus *Cupriavidus*. The isolate formed lytic zones with diameters of less than 10 mm (Table 1). *Cupriavidus* cells are gram-negative, peritrichously flagellated rods, non-sporulating, non-fermenting bacterium originally inhabiting soil. They were obtained from aquatic sediment in the oil palm plantation area. Their metabolism was oxidative, thus, they were found only in shallow river sediment. Several amino acids are their sole carbon and nitrogen sources.

The genus which resistance to various metals is widespread. The genus *Cupriavidus* is included in the biodiversity of various *Burkholderia cepacia*-like bacteria [20, 21]. They are beta-proteobacteria that have been identified as capable of establishing nitrogen-fixing nodules on legumes [22]. The isolate SBO06 could be a candidate for a biofertilizer for agriculture.

Family 1 included true lipase from bacteria, which is defined as a carboxylesterase that catalyzes the hydrolysis and synthesis of long-chain acylglycerols, with trioleoylglycerol being the standard substrate [23]. Subfamily 2 showed that expression in an active form of lipases depends on a chaperone protein named lipase-specific foldase, usually characterized by a slightly larger size (33 kDa) owing to an insertion in the amino acid sequence forming an anti-parallel, double-beta strand at the surface of the molecule [24]. Large-size molecules could have more difficulty crossing agar medium polymer; thus, their isolates might not be able to make large lytic zones in rhodamine B agar medium (Table 1).

The second cluster of phylogenetic tree consisted of isolates close to the genera *Serratia* and *Acinetobacter*. These were found only in sediment. *Serratia* isolates, SBF11 and SBF12, were found in forest sediments, and *Acinetobacter* isolates, SBO03, SBO04, SBO11, and

SBO12, were found in sediments in the oil palm plantation area. *Serratia* is a motile, gram-negative, rod-shaped, facultative anaerobe, commonly found in soil, water, air, plants, and animals. In this study, *Serratia* isolates were found only in aquatic sediment, because

they are facultative anaerobes. One characteristic of aquatic sediments is that they are micro-aerophilic, having close to semi-anaerobic conditions. *Serratia marcescens* releases an extracellular lipase and phospholipase into the medium [25]. The purified lipase
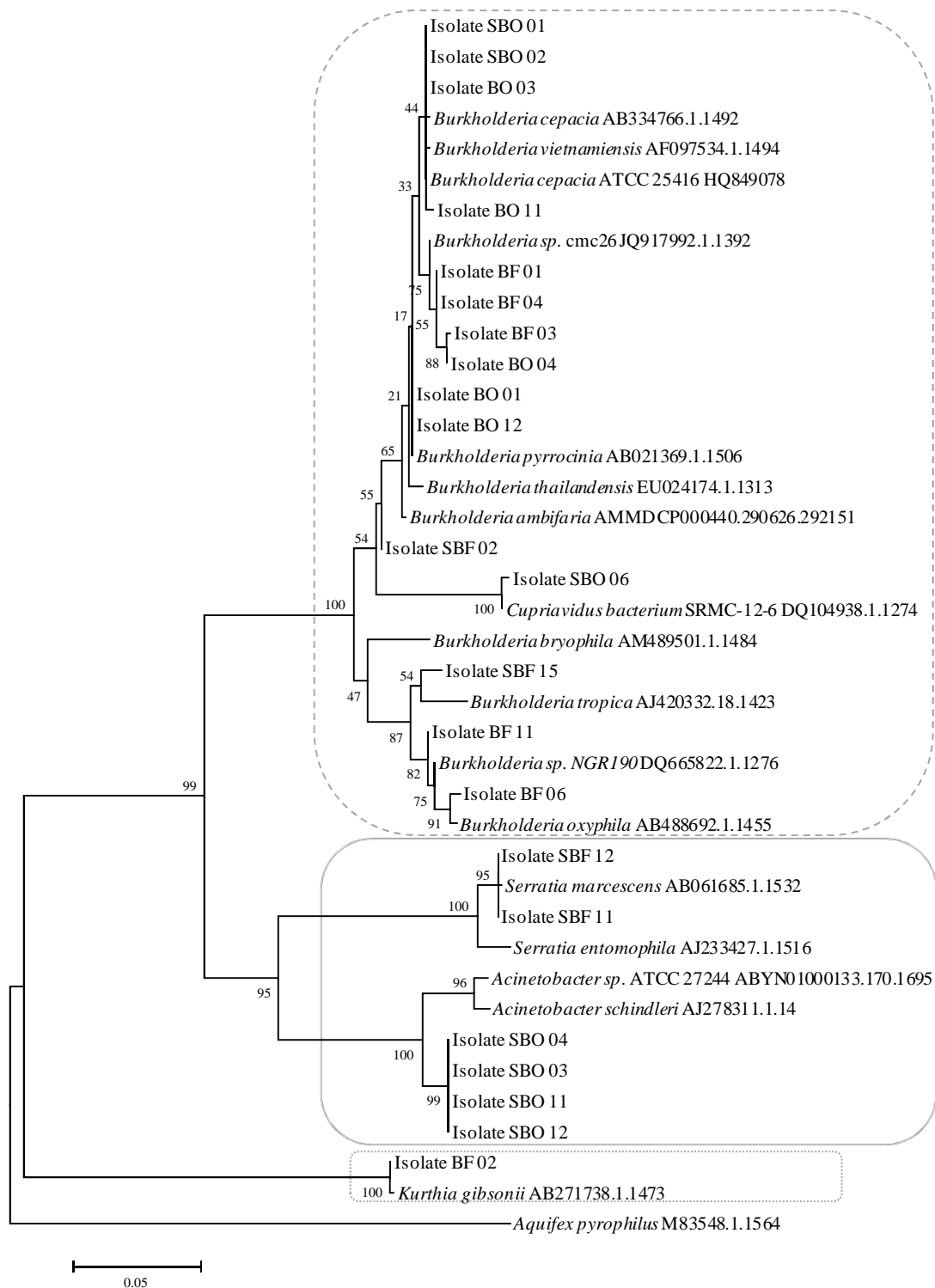

Figure 2. **Phylogenetic Tree Constructed by Maximum Likelihood Method Based on 16s rRNA Gene Sequences Data of 22 Lipolytic Bacterial Isolates**

is a dimer with two homologous subunits, of which the molecular mass is between 52–65 kDa, and the pI is 4.2. The optimal pH was shown to be pH 8–9, and the optimal temperature was shown to be 45 °C [26, 27]. In this study, the isolates SBF11 and SBF12 could form lytic zones of the greatest diameter (Table 1). Both isolates could be the best lipolytic bacteria candidates among our isolates.

Isolates SBO03, SBO04, SBO11, and SBO12, from oil palm plantation aquatic sediment, were phylogenetically close to the genus *Acinetobacter*. Organisms belonging to the genus *Acinetobacter* often are considered ubiquitous in nature, given that they can be recovered from almost all soil and surface water samples and can be pathogenic to human skin [28]. These isolates are gram-negative, catalase-positive, oxidase-negative, non-motile, non-fermenting coccobacilli. This genus has been known to be involved in biodegradation, leaching, and removal of several organic and inorganic, man-made hazardous wastes [29]. *Acinetobacter* has been reported that produces lipase and specific foldase with optimal acidity of pH 9–10, although they produce lipolytic enzymes of pH 4–8. Lipases of *Acinetobacter* have molecular masses of from 23 to more than 200 kDa [24, 26, 29]. Isolates SBO04 and SBO11 had lytic zones of from 7.5–10.25 mm (Table 1). The diversity of their molecular masses could account for their forming various diameters of lytic zones. The extracelullar enzyme of isolate SBO04 formed the essential fatty acids linoleic and arachidonic acid (Table 3). The isolate SBO11 formed eicosapentanoic acid (EPA). Every strain of *Acinetobacter* might have had different types of lipases; thus, their fatty-acid products could vary by type.

The third cluster of phylogenetic tree showed that isolate BF02, from forest soil, was closest to the genus *Kurthia* (Figure 2). This isolate is a gram-positive, aerobic, rod-class *Bacilli* family *Planococcaceae* [35]. The genus *Kurthia* originally was found in forest soil, rhizospheric soil of tea bushes, contaminated soil, sediment, water, and animals [31-34]. It could produce lipolytic enzymes, esterase lipase (C8) and esterase (C4), which are produced by *K. gibsonii* and *K. massilliensis*, although *K. zopfii* and *K. sibirica* could produce these enzymes weakly [35]. The isolate BF02 could not produce lipase for hydrolyzing long fatty acids to short fatty acids, but it formed longer fatty acids for an essential fatty-acid product. The genus *Kurthia* was a potential lipase-producing bacteria that produced a great quantity of lipases, like *Bacillus* [8].

Diversity of fatty acid production. Table 2 shows results of the GC-FID tests of supernatant separated from the bacteria biomass. Table 3 shows results for aquatic sediment lipolytic bacteria (all data not shown). Linolelaidic methyl ester (C18:2n6trans) dominated the

composition of fatty acids in the olive-oil substrate without lipolytic bacteria. All bacterial isolates showed diversity of fatty-acid products. Arachidic acid methyl ester (C20:0) was a fatty acid that dominated production of lipolytic bacteria from both the forest and the oil palm plantation. Isolate BO12 derived from oil palm plantation soil was closest to *Burkholderia* sp. (Figure 2). Isolates from soil and aquatic sediments contained fatty acids to at least 8 carbon chains (C8:0), including isolates BF04, BF06, BO03, BO04, BO12, SBO03, SBO06, SBO11, and SBO12. Those from the soil were close to the genera *Burkholderia,* and those from aquatic sediment, to *Acinetobacter*. The genera *Burkholderia* and *Acinetobacter* have been reported to have lipid metabolism for degrading and synthesizing various fatty acids [17, 36]. Lipolytic bacteria produce various fatty acids for arranging their cell structures, surviving environmental stress, and forming biofilm.

Essential fatty acids, such as linoleic acid (C18:2n6c), gamma linolenic acid (C18:3n6), arachidonic acid (C20:4n5), eicosa pentaenoic acid (C20:5n3), and docosahexaenoic acid (C22:6n3) were formed by isolates from both soil and aquatic sediment in both the forest and plantation area. Linoleic acids concentrations higher than substrate with olive oil (no lipolytic bacteria isolates) were contained in products of isolates BF06. Linoleic acid was produced by isolates BF01, BO01, BO12, and SBO04. The most linoleic acid was produced by isolate BO04. Isolates BF01, BO01, BO04, SBO04, and SBO11 from sediment. Most isolates were able to form EPA, except isolates from sediments, such as isolate SBF11. The highest EPA value was found in isolate SBO11, which was close to the genus *Acinetobacter*. The highest docosahexaenoic acid (DHA) value was produced by isolate SBF15, which

Table 2. Composition of Several Fatty Acids (µg/mg) Produced by Isolates of Lipolytic Bacteria from Topsoil

Code Sample	C18:2n6t	C18:2n6c	C18:3n6	C20:4n5	C20:5n3	C22:6n3
K*	3682.70	61.20	112.61	6.67	1.21	2.27
BF01	0.00	3196.52	6.11	29.06	2.00	5.82
BF02	2891.35	52.14	74.82	36.07	1.52	3.21
BF03	0.00	0.00	418.55	13.96	7.95	14.96
BF04	0.00	0.00	296.23	11.41	5.83	0.00
BF06	3899.10	85.00	109.72	60.25	1.49	1.39
BF11	0.00	0.00	465.52	0.00	7.87	15.84
BO01	0.00	4232.13	238.45	11.37	6.67	8.30
BO03	0.00	0.00	42.76	10.18	15.84	13.36
BO04	0.00	5701.54	293.28	2.55	5.53	10.00
BO11	0.00	0.00	12.80	14.79	5.29	2.52
BO12	0.00	3013.80	193.21	12.01	2.96	6.32

K* = substrate without lipolytic bacteria

Table 3. Composition of Several Fatty Acids (μg/mg) Produced by Isolates of Lipolytic Bacteria from Aquatic Sediment

Code Sample	C18:2n6t	C18:2n6c	C18:3n6	C20:4n5	C20:5n3	C22:6n3
K*	3682.70	61.20	112.61	6.67	1.21	2.27
SBF02	1334.50	23.50	45.27	36.55	1.17	3.88
SBF11	0.00	0.00	345.79	22.05	0.00	8.97
SBF12	0.00	0.00	625.45	60.92	8.93	16.82
SBF15	2614.09	46.96	70.65	43.22	1.34	19.55
SBO01	0.00	0.00	7.80	19.00	4.36	0.00
SBO02	0.00	0.00	389.03	17.51	6.48	10.81
SBO03	0.00	0.00	173.11	0.00	31.36	4.95
SBO04	0.00	2506.01	120.20	35.72	1.77	2.08
SBO06	1957.11	46.34	57.49	0.84	28.89	13.85
SBO11	1398.27	24.69	50.66	17.94	72.90	2.30
SBO12	27.96	0.49	1.01	0.36	1.46	0.05

K* = substrate without lipolytic bacteria

was close to the genus *Burkholderia*. Isolates BF04, BF06, SBO01, and SBO04 could not synthesize DHA. Isolate SBO06, which was closest to the genus *Cupriavidus*, could synthesize essential fatty acids, such as EPA and DHA. *Cupriavidus pauculus* has been reported to be able to produce true lipase (C14), ester lipase (C8), and esterase (C4), but others could not produce all three kinds of lipolytic enzymes simultaneously [19]. They produced catalase and oxidase to protect EPA and DHA from oxidative attack.

Polyunsaturated fatty acids (PUFAs) play an increasingly important role as biomedical and nutraceutical agents. Many of them belong to the essential fatty acids, uptake of which is required for membrane-lipid and prostaglandin synthesis. Microbial lipases are used to enrich PUFAs from animal and plant lipids, such as "menhaden," tuna, or borage oil. Free PUFAs and their mono- and diglycerides are used to produce a variety of pharmaceuticals, including anti-cholesterol-emics, anti-inflammatories, and thrombolytics [37]. Bacterial lipases can discriminate against Omega-3 PUFAs, such as EPA and DHA, and, hence, lipase-catalyzed hydrolysis has been used for production of Omega-3 PUFA concentrates [38].

The diversity of lipases produced from bacterial isolates shows that lipolytic bacteria cannot hydrolyze long fatty acids to short fatty acids, but they can form essential fatty acids with longer acylglycerol and longer fatty acid methyl ester (Tables 2-3). Bacterial lipases have been reported that could synthesize important organic compounds. The lipase from *B. cepacia* is a catalyst in organic synthesis for the kinetic resolution of racemic

mixtures of secondary alcohols in hydrolysis, esterification, and trans-esterification [26]. Their capabilities can be used in several industries for food, feed, and bioenergy.

4. Conclusions

Lipolytic bacteria isolated from soil and aquatic sediment in a forest and on an oil palm plantation at Sarolangun District, Jambi Province, Indonesia taxonomically belong to five genera and phylogenetically, are distributed in three groups: the *Burkholderia-Cupriavidus* group, the *Serratia-Acinetobacter* group, and the *Kurthia* group. Their lipolytic enzymes formed various fatty acids. Some isolates formed essential fatty acids, such as linoleic, linolenic, arachidonic, EPA, and DHA. In future, lipolytic bacteria can be used for agro-industry and aquaculture.

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